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(54) **METHOD OF IN VIVO SCREENING OF THERAPEUTIC AGENT FOR MEMORY/LEARNING DYSFUNCTION BY SCHIZOPHRENIA**

(57) A method of evaluating memory/learning functions with the use of a model with glutamic acid N-methyl-D-aspartate (NMDA) type receptor hypofunction as an animal model for schizophrenia and with the use of reference memory task, wherein there has been found concrete means for detecting any differences in activity be-

tween typical anti-psychosis drugs and atypical anti-psychosis drugs is found.

An in vivo animal model for screening of a therapeutic agent for improving cognitive dysfunction by schizophrenia is provided.

## Description

## TECHNICAL FIELD

5 **[0001]** The present invention relates to an in vivo screening method of a therapeutic agent for improving memory/learning dysfunctions by schizophrenia.

## BACKGROUND ART

10 **[0002]** Glutamic acid is a most popular excitatory neurotransmitter in the central nervous system, and the receptors thereof are classified broadly into an NMDA type, an  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) type, a kainate type, and a metabotropic type. It is revealed that the NMDA type receptor plays an important role in the completion of the long-term potentiation (LTP), which is an electrophysiologically basal process of the memory/learning functions (cf., Science 285: 1870-1874 (1999)). At the animal level, it is known that an NMDA receptor antagonist may  
 15 induce memory/learning dysfunctions in various memory/learning tasks such as a passive avoidance response, a radial maze, a T or Y maze, a water maze, a place or object recognition, an autoshaping learning task, and a lever-pressing task (cf., Brain Res Rev 41: 268-287 (2003)). It is also reported that PCP or ketamine, which is also an NMDA receptor antagonist, induces cognitive dysfunctions in humans (cf., Psychopharmacology 169: 215-233 (2003)). Namely, it is a common opinion among the electrophysiological level, the animal level and the human level that the NMDA type receptor  
 20 plays an important rule in the memory/learning process.

**[0003]** It is the most widely-accepted hypothesis that the hypofunction of the NMDA type receptor is regarded as a mechanism of development of schizophrenia. This hypothesis is established based on the following four points:

25 (i) PCP and ketamine, which are an NMDA receptor antagonist, induce the major symptoms of schizophrenia in normal humans including cognitive dysfunction, positive negative symptoms, and induce the exacerbation of symptoms in patients with schizophrenia (cf., Am J Psychiatry 158: 1367-1377 (2001), Psychopharmacology 169: 215-233 (2003)):

30 (ii) There are clinical reports that glycine, D-serine and D-cycloserine, which have been known to elevate NMDA receptor functions, enhance the drug efficacy of anti-psychotic drugs in patients with schizophrenia, and improve negative symptoms and cognitive dysfunctions (cf., Am J Psychiatry 158: 1367-1377 (2001), Psychopharmacology 169: 215-233 (2003)):

35 (iii) The variation in the amount of glutamic acid per se, or the amount of the endogenous substance: N-acetyl-aspartyl glutamate (NAAG) having an NMDA antagonistic activity is observed in patients with schizophrenia (cf., Am J Psychiatry 158: 1367-1377 (2001)), and the change in the amount of mRNA and proteins of NMDA type receptor subunit and NMDA receptor-related protein is observed in patients with schizophrenia (cf., Am J Psychiatry 157: 1811-1823 (2000), Am J Psychiatry 160: 1100-1109 (2003), Society for Neuroscience Program No. 754.4. (2003)):

40 (iv) A series of genes which were found as a gene relating to the onset of schizophrenia (Neuregulin 1, G72, dysbindin, calcineurin) are genes being capable of modifying NMDA receptor functions (cf., Proc Natl Acad Sci USA 99: 13365-13367 (2002), Neuron 40: 881-884 (2003)). From the above facts, it is generally considered that the dysfunctions induced by NMDA type receptor antagonists are models to reflect the dysfunctions by schizophrenia.

45 **[0004]** Schizophrenia is associated with various cognitive dysfunctions such as attention, memory, learning, executive functions, but it is reported that among these functions, especially a certain memory function is selectively and seriously damaged. Namely, memory is classified broadly into procedural memory and declarative memory. The declarative memory is further classified into short-term memory/working memory and a long-term memory/reference memory. It is reported that in schizophrenia, the declarative memory including both of the working memory and the reference memory is selectively damaged, and further, among them, the damage of the reference memory is most serious. Recently, it is reported that the cognitive dysfunction including such a reference memory dysfunction is the most important predictive  
 50 factor of social daily ability and professional ability, and a quality of life of patients with schizophrenia. Then, at the moment, the cognitive dysfunction is positioned as a core symptom of schizophrenia (cf., Psychopharmacology 169: 213-214 (2003), Proc Natl Acad Sci USA 96: 13591-13593 (2002), Psychoneuroendocrinology 28: 27-38 (2003), Psychiat Clin N Am 26: 25-40 (2003)). Under these circumstances, many clinical trials are being done with respect to the effects of the existing anti-psychotic agents on various cognitive dysfunctions by schizophrenia (cf., Psychopharmacology 162: 11-17 (2002), Psychoneuroendocrinology 28: 27-38 (2003), Psychiat Clin N Am 26: 25-40 (2003)), and there is being submitted an evidence that a typical anti-psychotic agent, haloperidol, is ineffective, while some of atypical anti-psychotic agents are effective (cf., J. Clin Psychiatry 65: 361-372, Psychoneuroendocrinology 28: 27-38 (2003), Psychiat Clin N Am 26: 25-40 (2003)). However, the drug efficacy of these existing drugs are not sufficient enough, and hence, it has

been discussed that it is important to develop a therapeutic agent for cognitive dysfunctions by schizophrenia (cf., Science 299: 350-351 (2003)).

[0005] On the other hand, in the research and development of therapeutic agents for human diseases, it is generally essential to develop an animal model being suitable for screening thereof. In such an animal model, the face validity (similarities of symptoms), the construct validity (similarities of the mechanism of development of symptoms), and the predictive validity (predictability of clinical drug efficacy), and further, the easiness being suitable for screening are required. However, it is considered that an animal model for cognitive dysfunctions by schizophrenia satisfying such requirements is quite limited at the moment. Namely, as an animal model for schizophrenia being capable of satisfying the above-mentioned requirements, PCP-induced models showing prepulse inhibition or social interaction failure can be exemplified (cf., Prog Neuropsychopharmacol Biol Psychiatry 27: 1071-1079 (2003)). It is known that the drug efficacy of atypical anti-psychotic agents, but that of a typical anti-psychotic agent can be selectively detected in these models, and the results obtained in these animal models partially reflect the clinical effects of a drug on schizophrenia. However, it is considered that among these animal models the former one may reflect the disorder of the sensorimotor gating function in schizophrenia, while the latter may reflect the negative symptoms of schizophrenia such as social withdrawal. Thus, as mentioned above, an easy model for evaluating drug efficacy, (1) being capable of reflecting reference memory dysfunction, which is the most serious cognitive dysfunction in schizophrenia (i.e., face validity, similarities of symptoms); (2) being associated with NMDA receptor hypofunction, which is a most possible cause for schizophrenia (i.e., construct validity, similarities of the mechanism of onset); and (3) being capable of detecting an excellent drug efficacy of an atypical anti-psychotic agent rather than that of a typical anti-psychotic agent (predictive validity, predictability of clinical drug efficacy), is considered to be quite useful in the research and development of a therapeutic agent for cognitive dysfunction, a core symptom of schizophrenia, but such an animal model has not been known yet until now.

[0006] In animals, various tasks consisting of both of the training session, and the testing session being carried out after a prescribed period from the testing session, can be used in order to study a reference memory. In the training session, the animals are made to learn an avoidance response such as electroconvulsive shock (passive or active avoidance response), a task of reaching to a platform which is not visible below water (a water maze task), a task of fetching a food after getting through a maze task or a task of avoiding an electroconvulsive shock (radial maze task, Y or T maze task), a task of recognizing and searching a novel place or object, a task of pressing a lever for obtaining a food, etc., and further they are made to acquire the memory thereof. The animals are returned to exactly the same experimental environment after a prescribed period therefrom, and they are tested if they can retrieve the acquired memory. In the reference memory tasks, the animals can memorize all of the specific environment and stimulus which are given to them in a specific order in the training session. In order to correctly evaluate at the testing session the success and failure of the memory acquisition in the training session, it is necessary to carry out the training session and the learning session under exactly the same environment and stimulus. Especially, there is a report that animals show an abnormal memory retrieval when ethanol or an NMDA type receptor antagonist is administered only in the training session of the reference memory tasks, but not in the testing session (cf., Brain Res 706: 227-232 (1996), Pharmacol Biochem Behav 69: 585-593 (2001)). In such cases, the animals acquire a reference memory depending on the environment in the brain, which is induced by the administration of a drug, and in fact, it is proved that by administering a drug both in the training session and the testing session, the acquired memory can correctly be retrieved. Such a phenomenon is usually called state-dependency. In the evaluation of the reference memory functions, it is sometimes necessary to give sufficient consideration to the state-dependency in some cases, and it is necessary to avoid any artificial misjudgment on the evaluation of memory functions due to state-dependency. As mentioned above, in order to avoid any state-dependency effect by an agent for inducing memory/learning dysfunctions such as an NMDA type receptor antagonist, and to evaluate the reference memory/learning function, there is a method comprising administering an agent for inducing memory/learning dysfunctions in both of the training session and the testing session. Further, as an alternative method, it is easily speculated to utilize a method comprising chronically administering an agent for inducing memory/learning dysfunctions during the period including the training session and the testing session. In addition, instead of administering an agent for inducing memory/learning dysfunctions, it may be possible to utilize a method comprising expressing a chronic dysfunction during the period including the training session and the testing sessions of the reference memory tasks with the use of a gene engineering technique. For example, an NMDA type receptor subtype NR1 knock-down or NR2A subtype knockout animal are already produced as a concrete example for an animal model showing a chronic hypofunction of NMDA type receptor (cf., Cell 98: 427-436 (1999), J Neurosci 21: 750-757 (2001)).

#### DISCLOSURE OF INVENTION

[0007] The present invention provides a screening method of a therapeutic agent for memory/learning dysfunctions by schizophrenia. More particularly, the present invention provides an animal model for reference memory dysfunction caused by hypofunction of NMDA type receptor as a simple animal model for schizophrenia providing the predictability of the clinical drug efficacy of the existing therapeutic agents.

[0008] The present inventors have intensively studied in order to solve the above problems, and found that the reference memory dysfunction in the animals where NMDA type receptor hypofunction is induced both in the training session and the testing session is specifically improved by an atypical anti-psychotic agent but not by a typical anti-psychotic agent, and further they have confirmed that this evaluation system is a very simple and highly-reproducible evaluation system, and finally they have accomplished the present invention.

[0009] Namely, the present invention relates to the following features:

[1] An in vivo screening method for predicting whether or not a test compound is capable of improving the memory/learning dysfunctions by schizophrenia, wherein said method comprises a step of evaluating the memory/learning functions by employing a model showing glutamic acid N-methyl-D-aspartate (NMDA) type receptor hypofunction as an animal model for schizophrenia, and a reference memory task.

[2] The method according to the above [1], wherein the reference memory task is a passive avoidance task, an active avoidance task, a water maze task, a radial maze task, a T or Y maze task, a place recognition task, an object recognition task, an autoshaping learning task, or a lever-pressing task.

[3] The method according to the above [1], wherein the reference memory task is composed of two sessions of training and testing, and in the training session the animals are made to learn either of the tasks described in the above [2] and to acquire the memory of said task, and in the testing session being carried out after a prescribed period from the training session, the retention and retrieval ability of said memory of the animals are quantified.

[4] The method according to the above [1], wherein the model showing an NMDA type receptor hypofunction is produced by administering a compound having an NMDA type receptor antagonistic activity, e.g., MK-801, phencyclidine (PCP), ketamine, or a derivative thereof, to the animals in both of the training session and the testing session of the reference memory task, or by chronically administering said compound or a derivative thereof to the animals during the period including the training session and the testing session.

[5] The method according to the above [1], wherein the model showing an NMDA type receptor hypofunction is an animal model associated with an NMDA type receptor hypofunction due to variation, overexpression, or deficiency of gene of constitutive proteins or relevant proteins of an NMDA type receptor in both of the training session and the testing session of the reference memory task.

[6] A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises a substance selected by a screening method as set forth in any one of the above [1] to [5] as an active ingredient.

[7] A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises a serotonin 5-HT<sub>1A</sub> antagonist selected by a screening method as set forth in any one of the above [1] to [5] as an active ingredient.

[8] A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises a choline acetylase inhibitor selected by a screening method as set forth in any one of the above [1] to [5] as an active ingredient.

[9] A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises aricept as an active ingredient.

[10] A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises quetiapine as an active ingredient.

[11] The therapeutic agent for the memory/learning dysfunctions by schizophrenia according to the above [10], which comprises as an active ingredient quetiapine in a daily dose of 5 to 270 mg.

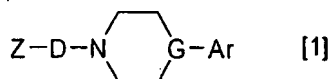
[12] The therapeutic agent for the memory/learning dysfunctions by schizophrenia according to the above [10], which comprises as an active ingredient quetiapine in a daily dose of 15 to 90 mg.

[13] A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises clozapine as an active ingredient.

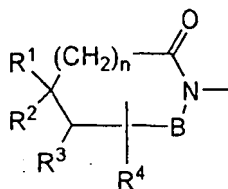
[14] The therapeutic agent for the memory/learning dysfunctions by schizophrenia according to the above [13], which comprises as an active ingredient clozapine in a daily dose of 0.2 to 34.5 mg.

[15] The therapeutic agent for the memory/learning dysfunctions by schizophrenia according to the above [13], which comprises as an active ingredient clozapine in a daily dose of 0.7 to 11.5 mg.

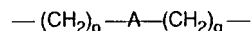
[16] A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises as an active ingredient an imide derivative of the formula [1]:



{wherein Z is a group of the formula:



(in which B is a carbonyl or a sulfonyl; R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are independently a hydrogen atom or a lower alkyl, provided that R<sup>1</sup> and R<sup>2</sup>, or R<sup>1</sup> and R<sup>3</sup> may combine each other to form a hydrocarbon ring, or R<sup>1</sup> and R<sup>3</sup> may combine each other to form an aromatic hydrocarbon ring; said hydrocarbon ring may optionally be cross-linked with a lower alkylene or an oxygen atom; said lower alkylene and hydrocarbon ring may optionally be substituted by at least one alkyl; and n is 0 or 1), D is a group of the formula:



(in which A is a hydrocarbon ring optionally be cross-linked with a lower alkylene or an oxygen atom; said lower alkylene and said hydrocarbon ring may optionally be substituted by at least one alkyl; and p and q are independently 0, 1 or 2),

G is N, CH or COH, and -Ar is an aromatic heterocyclic group, an aromatic hydrocarbon group, benzoyl, phenoxy, or phenylthio, or

G is a carbon atom, and -Ar is a biphenylmethylidene,

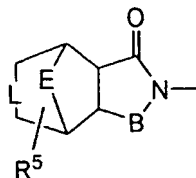
where said aromatic heterocyclic group, aromatic hydrocarbon group, benzoyl, phenoxy, phenylthio, and biphenylmethylidene may optionally be substituted by at least one group selected from a lower alkyl, a lower alkoxy and a halogen atom),

or an acid addition salt thereof.

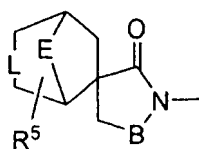
[17] The therapeutic agent for the memory/learning dysfunctions by schizophrenia comprising as an active ingredient the imide derivative or an acid addition salt thereof according to the above [16], wherein Ar is an aromatic heterobicyclic group, naphthyl, benzoyl, phenoxy or phenylthio, and G is N, CH or COH, or -Ar is a biphenylmethylidene, and G is a carbon atom (said aromatic heterobicyclic group, naphthyl, benzoyl, phenoxy, phenylthio and biphenylmethylidene may optionally be substituted by at least one group selected from a lower alkyl, a lower alkoxy and a halogen atom).

[18] The therapeutic agent for the memory/learning dysfunctions by schizophrenia comprising as an active ingredient the imide derivative or an acid addition salt thereof according to the above [16], wherein Ar is an aromatic heterocyclic group condensed with a benzene ring, or naphthyl, benzoyl, phenoxy or phenylthio (said aromatic heterocyclic group condensed with a benzene ring, naphthyl, benzoyl, phenoxy, and phenylthio may optionally be substituted by at least one group selected from a lower alkyl, a lower alkoxy and a halogen atom), and G is N, CH or COH.

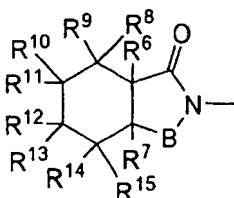
[19] The therapeutic agent for the memory/learning dysfunctions by schizophrenia comprising as an active ingredient the imide derivative or an acid addition salt thereof according to the above [16], wherein Z is a group of the formula:



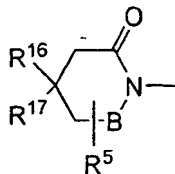
(in which -L- is a single bond or a double bond, E is a lower alkylene optionally substituted by a lower alkyl, or an oxygen atom, R<sup>5</sup> is a hydrogen atom or a lower alkyl, and B is the same as defined in the above [14]); a group of the formula:



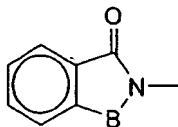
(in which -L-, E, R<sup>5</sup> and B are as defined above);  
a group of the formula:



(in which R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup> are independently a hydrogen atom or a lower alkyl, or the adjacent two groups of R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup> may combine each other to form a double bond, and B is as defined above);  
a group of the formula:

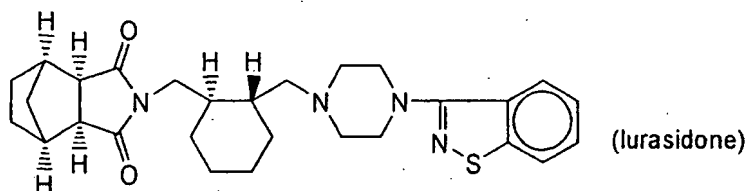


(in which R<sup>16</sup> and R<sup>17</sup> are independently a hydrogen atom or a lower alkyl, or R<sup>16</sup> and R<sup>17</sup> may combine each other to form a saturated hydrocarbon ring, and R<sup>5</sup> and B are as defined above); or  
a group of the formula:

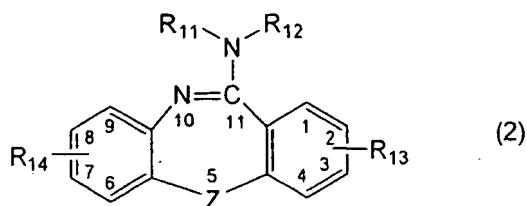


(in which B is as defined above).

[20] A therapeutic agent for the memory/learning dysfunctions by schizophrenia comprising as an active ingredient the imide derivative or an acid addition salt thereof, wherein the compound of the formula [1] is lurasidone:



[21] A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises as an active ingredient a compound of the formula (2):



10 wherein Z is a divalent sulfur, imino, or lower alkylimino;

R<sub>11</sub> is a hydrogen atom or an alkyl having 1 to 5 carbon atoms;

R<sub>12</sub> is a hydrogen atom, an alkyl having 1 to 5 carbon atoms, a phenyl, an R<sub>15</sub>-substituted phenyl, an aminoalkyl having 1 to 5 carbon atoms, a lower alkylaminoalkyl having 2 to 8 carbon atoms, a lower alkylamino, an amino, or a lower alkylamino; or

15 R<sub>11</sub> and R<sub>12</sub> may combine each other together with N to form a 1-pyrrolidinyl, piperidino, morpholino, thiomorpholino, 1-piperazinyl, a 4-lower alkyl-1-piperazinyl, a 4-(hydroxy-lower alkyl)-1-piperazinyl or a 4-(lower alkoxy-lower alkyl)-1-piperazinyl; and

R<sub>13</sub>, R<sub>14</sub>, and R<sub>15</sub> are independently a hydrogen atom, a halogen atom, a hydroxy group, a trifluoromethyl, a lower alkyl, a lower alkoxy, or a lower alkylthio,

20 or an acid addition salt thereof.

[0010] The present invention provides a concrete method for evaluating a memory/learning improving activity being specific to certain atypical anti-psychotic agents but not to a typical anti-psychotic agent haloperidol. The results obtained by this method are in agreement with the clinical findings that haloperidol shows no improving activity of cognitive dysfunctions, and that atypical anti-psychotic agents exhibit an improving activity of cognitive dysfunctions. As a result, it becomes possible to provide a screening method of a therapeutic agent for cognitive dysfunctions by schizophrenia, and further provides a concrete therapeutic agent therefor. Actually, by the present method, the memory/learning improving efficacy is recognized with respect to lurasidone, which is under development as a candidate for a novel therapeutic agent for schizophrenia, noradrenaline  $\alpha$ 2 receptor antagonist 1-(2-pyrimidyl)piperazine dihydrochloride (hereinafter, referred to as 1-PP), and serotonin 5-HT 1A receptor antagonist in addition to risperidone, clozapine or quetiapine, by which a candidate for a novel agent for improving cognitive dysfunctions by schizophrenia can be provided. In addition, the memory/learning improving activity of clozapine and quetiapine is observed at a dose by 10 times or more lower than the dose at which they exhibit an anti-psychotic activity, and hence, a different action mechanism can be speculated. Further, since it is suggested that the retrieval of reference memory task being acquired with improvement by a drug is done with depending on the state of the NMDA type receptor hypofunction (state-dependent), as a novel and concrete method for evaluating the reference memory dysfunction improving activity of a drug, a method where the NMDA type receptor hypofunction is induced during the period including the training session and the testing session is effective.

#### BEST MODE FOR CARRYING OUT THE INVENTION

40 [0011] The present invention relates to an in vivo screening method for predicting whether or not a test compound is capable of improving the memory/learning dysfunctions by schizophrenia, wherein said method comprises a step of evaluating the memory/learning function by employing a model showing an NMDA type receptor hypofunction as an animal model for schizophrenia and a reference memory task.

[0012] The reference memory task includes, for example, a passive avoidance task, an active avoidance task, a water maze task, a radial maze task, a T or Y maze task, a place recognition task, an object recognition task, an autoshaping learning task, and a lever-pressing task.

[0013] The reference memory task is composed of two sessions such as training and testing. In the training session, an animal is made to learn a certain task and further made to acquire a memory thereof. After a prescribed period from the learning session, the testing session is carried out, and the retention and retrieval of the memory of task at the testing session is quantified.

[0014] A compound having an NMDA type receptor antagonistic activity, e.g., MK-801, PCP, ketamine, or a derivative thereof, is administered to animals during both in the training session and the testing session of the reference memory task, or chronically administered to animals during the period including the training session and the testing session, and these animals are used as an animal model showing an NMDA type receptor hypofunction.

55 [0015] Alternatively, an animal model associated with an NMDA type receptor hypofunction due to variation, overexpression or deficiency of gene of constitutive proteins or relevant proteins of NMDA type receptor in both of the training session and the testing session in the reference memory task can be used as a model showing an NMDA type receptor hypofunction.

**[0016]** Hereinafter, the present invention is illustrated in more detail by Examples, but the present invention should not be construed to be limited thereto.

#### Example 1

##### (Method)

**[0017]** Wistar male rats (7 weeks old) were used. Haloperidol (a typical anti-psychotic agent), clozapine, quetiapine, risperidon, olanzapine or aripiprazole (atypical anti-psychotic agent), or lurasidone being under development as a novel anti-psychotic agent was suspended in a 0.5 % methyl cellulose (MC) and the resultant suspension was used as a test compound. Serotonin 5-HT<sub>1A</sub> receptor antagonist WAY-100635 or noradrenaline  $\alpha_2$  receptor antagonist 1-PP was dissolved in a physiological saline solution (Otsuka Pharmaceutical Co., Ltd.) and used as a test compound. As an NMDA type receptor antagonist, MK-801 hydrogen maleate (SIGMA-ALDRICH M-107) was dissolved in a physiological saline solution (Otsuka Pharmaceutical Co., Ltd.). A test compound (0.3 to 10 mg/kg) or a 0.5 % MC or a physiological saline solution as a control was orally or interperitoneally administered to the animals one hour prior to the training session of the passive avoidance task, and MK-801 (0.05 mg/kg) or a physiological saline solution as a control was subcutaneously administered 30 minutes prior to both of the training session and the testing session. For the evaluation of state-dependency of MK-801, MK-801 was administered before the training session, and a physiological saline solution was administered before the testing session instead. The dosing value thereof was 5 ml/kg for each.

**[0018]** The step-through type passive avoidance response test was carried out in the following manners with the use of an apparatus consisting of a light-dark box, a slide door dividing the light-dark box and a shock generator (manufactured by O'hara & Co., Ltd., PA-2030A, PA-300 1A) as an experimental apparatus. Namely, on Day 1 of the experiment, after a test compound and MK-801 were administered, the rats were put into the light box of the experimental apparatus where the back of each rat was directed to the dark box. Then, 10 seconds later, a slide door set at the border between the dark box and the light box was opened. Due to the habits of the rats, once the rats entered into the dark box, the slide door was quickly closed. At three seconds after entering into the dark box, an electroconvulsive shock (0.5 mA, for 3 seconds) was given to the rats. The period between the time just after the slide door was opened and the time at which the rats entered into the dark box was measured as a step-through latency. As to the animals which did not enter into the dark room even after 300 seconds, the training was terminated, and those animals were dropped in the following experiment for the reasons of training failure. On Day 2 of the experiment, the testing session was carried out about 24 hours after the training session. Thirty minutes prior to the testing session, MK-801 or a vehicle thereof, i.e., a physiological saline solution, was subcutaneously administered to the rats. The procedures of the testing session were carried out in the same manner to the training session except that no electroconvulsive shock was given. The step-through latency at the testing session was measured up to 300 seconds, and the step-through latency over 300 seconds was regarded as 300 seconds. The number of animals which showed 300 seconds at the testing session was counted, and the ratio thereof was calculated in percentages (as defined as a % of animals avoiding) in each group. The statistical analysis of the % of animals avoiding was done by  $\chi^2$  test with Bonferroni's correction. The animals were used in a group of 10 to 25 animals per group, and the data was expressed by percentage.

##### (Results)

**[0019]** First, a drug was administered alone without MK-801, and a dose of the drug to induce the memory/learning dysfunctions in the passive avoidance response was determined. Further a dose less than that dose and to induce no memory/learning dysfunctions was used as an administration dose in the MK/801 models (Table 1).

**[0020]** The animals to which MK-801 was administered before the training session, and a physiological saline solution was administered before the testing session showed the nearly equal decrease in the step-through latency to that of the animals to which MK-801 was administered both in the training session and the learning session. That is, it was found that the memory dysfunction observed in the cases where MK-801 was administered only before the training session, or before both of the training session and the testing sessions, is not a memory retrieval dysfunction but memory acquisition dysfunction.

**[0021]** Next, the effects of drugs on the memory dysfunction observed in the cases where MK-801 was administered in both of the training session and the testing session were studied. In the group to which MK-801 was not administered, the animals showing 300 seconds of step-through latency (% of animals avoiding) was 75 to 80 % in all of the experiments. On the contrary, the % of animals avoiding became 0 to 5 % in all of the experiments by the administration of MK-801. The effects of the test compounds on the % of animals avoiding were shown in Table 1. As is shown in Table 1, there were differences of the drug efficacy strength among individual drugs. That is, while no drug efficacy was observed with respect to either a typical anti-psychotic agent haloperidol, or atypical anti-psychotic agents olanzapine, aripiprazole, but a significant improving activity was observed with respect to atypical anti-psychotic agents clozapine, quetiapine,



lurasidone, and serotonin 5-HT 1A receptor antagonist WAY-100635, noradrenalin  $\alpha$ 2 receptor antagonist 1-PP.

[0022] Interestingly, it is known that a dose to inhibit 50 % of methamphetamine-induced hyperlocomotion in rats (ED<sub>50</sub>, mg/kg, p.o.), which reflects anti-psychotic activity, is 65 and about 100 with respect to clozapine and quetiapine, respectively. That is, it is suggested that clozapine and quetiapine show a memory/learning dysfunction improving activity at a dose 65 -272 times or 10 times lower than the dose thereof showing anti-psychotic activity, ED<sub>50</sub>, respectively. Thus, it became apparent that these drugs show its memory/learning dysfunction improving activity in a different action mechanism from that of the anti-psychotic activity thereof. Since the clinical daily dose of clozapine as an anti-psychotic agent is 150-750 mg, then a dose thereof for improving memory/learning dysfunctions by schizophrenia should be 0.2-34.5 mg, preferably 0.7-11.5 mg, which are obtained by calculating 150/217-750/65 (i.e., 0.7-11.5 mg) and broadening the obtained range into either direction by about 3 times. On the other hand, since the clinical daily dose of quetiapine as an anti-psychotic agent is 150-900 mg, then a dose thereof for improving memory/learning dysfunctions by schizophrenia should be 5 - 270 mg, preferably 15 - 90 mg, which is obtained by calculating 150/10-900/ 10 (i.e., 15-90 mg) and broadening the obtained range into either direction by about 3 times.

[0023] Finally, with respect to lurasidone and 1-PP, which showed the most remarkable memory/learning dysfunction improving activity, when the effects thereof on the memory dysfunction in cases where MK-801 was administered only in the training session were studied, either of these drugs showed no drug efficacy. From these results, it was proved that the memory of passive avoidance response being acquired with improvement by lurasidone and 1-PP depends on the state of MK-801 administration, i.e., state-dependent.

#### Example 2

[0024] In the procedures of Example 1, PCP HCl (0.75 mg/kg) was subcutaneously administered instead of MK-801 (0.05 mg/kg) to animals prior to both of the training session and the testing session of the memory/learning task to induce memory/learning dysfunctions, and the memory/learning dysfunction improving activity of a test compound can be evaluated.

#### Example 3

[0025] The memory/learning dysfunction improving activity of a test compound can be evaluated under the exactly same conditions as those in Examples 1 and 2, except that ketamine is used instead of MK-801 or PCP HCl.

#### Example 4

[0026] In the procedures of Examples 1 to 3, instead of subcutaneous administration of an NMDA type receptor antagonist such as MK-801, PCP HCl or ketamine prior to both of the training session and the testing session, these drugs are injected to an ALZET osmotic pump and implanted under the skin of the back side of the animals so that these drugs are chronically administered under the skin during the period including the training session and the testing session, and a memory/learning dysfunctions can be induced thereby, and then the memory/learning dysfunction improving activity of a test compound thereon can be evaluated.

#### Example 5

[0027] Instead of the method of using an NMDA type receptor antagonist as shown in the above Examples, an animal showing a variation, overexpression or deficiency of gene of constitutive proteins or relevant proteins of the NMDA type receptor can be utilized.

#### Example 6

[0028] In the procedures of Examples 1 to 5, either one of an active avoidance task, a water maze task, a radial maze task, a T or Y maze task, a place recognition task, an object recognition task, an autoshaping learning task, and a lever-pressing task can be employed instead of a passive avoidance task, and the exactly same experiment as Example 1 can be carried out.

[0029] Table 1 shows the effects of the drugs on MK-801-induced memory/learning dysfunctions in the passive avoidance task in rats.

[0030] To be more precise, Table 1 shows the effects of the drugs in terms of the percentage of the animals acquiring passive avoidance response (i.e., the percentage of the animals showing a step-through latency of 300 seconds or more in the testing session, that is, the percentage of the animals showing a remarkable memory retention, a % of animals avoiding) in the passive avoidance response test in the rats to which MK-801, an NMDA type receptor antagonist, was

administered prior to both of the training session and the testing session.

[0031] In a similar passive avoidance response test, a minimum dose of a drug to induce memory/learning dysfunctions by a single application thereof is shown in the right edge column of Table.

[0032] The figures in Table mean a % of animals avoiding. In a similar passive avoidance response test, a minimum dose of a drug to induce memory/learning dysfunctions by a single application thereof is shown in the right edge column of Table.

Table 1

Drug	Vehicle group	Group treated with MK-801 (0.05 mg/kg, s.c.)	Groups treated with MK-801+ Drug (mg/kg, p.o.)					Minimum dose to induce memory dysfunction by single application, mg/kg, p.o
			0.1	0.3	1	3	10	
(MK-801 administered prior to training session)								
Lurasidone	80	0	-	20	20	30	20	>30
1-PP	92	0	-	-	-	8	-	-
(MK-801 administered prior to both of training and testing sessions)								
Haloperidol	80	0	0	5	15	10	-	10
Olanzapine	80	0	-	15	20	20	10	3
Risperidone	76	4	20	20	28	10	-	3
Clozapine	80	4	-	44*	36*	20	10	10
Quetiapine	75	5	-	10	20	30	50*	30
Aripiprazole	80	4	-	-	16	16	-	10
Lurasidone	80	0	-	40*	35*	75*	70*	>30
1-PP	80	13	-	-	47*	67*	-	-
WAY-100635	75	5	-	30	40*	30	-	-

n=10-25

\*P<0.05 vs Group treated with MK-801 (0.05 mg/kg s.c.)

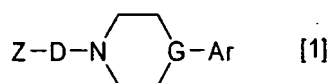
#### INDUSTRIAL APPLICABILITY

[0033] The present invention provides an in vivo screening method of a therapeutic agent for improving memory/learning dysfunctions by schizophrenia.

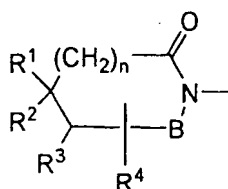
#### Claims

1. An in vivo screening method for predicting whether or not a test compound is capable of improving the memory/learning dysfunctions by schizophrenia, wherein said method comprises a step of evaluating the memory/learning functions by employing a model showing glutamic acid N-methyl-D-aspartate (NMDA) type receptor hypofunction as an animal model for schizophrenia, and a reference memory task.
2. The method according to claim 1, wherein the reference memory task is a passive avoidance task, an active avoidance task, a water maze task, a radial maze task, a T or Y maze task, a place recognition task, an object recognition task, an autoshaping learning task, or a lever-pressing task.
3. The method according to claim 1, wherein the reference memory task is composed of two sessions of training and testing, and in the training session the animals are made to learn either of the tasks described in claim 2 and to acquire the memory of said task, and in the testing session being carried out after a prescribed period from the training session, the retention and retrieval ability of said memory of the animals are quantified.

4. The method according to claim 1, wherein the model showing an NMDA type receptor hypofunction is produced by administering a compound having an NMDA type receptor antagonistic activity, e.g., MK-801, phencyclidine (PCP), ketamine, or a derivative thereof, to the animals in both of the training session and the testing session of the reference memory task, or by chronically administering said compound or a derivative thereof to the animals during the period including the training session and the testing session.
5. The method according to claim 1, wherein the model showing an NMDA type receptor hypofunction is an animal model associated with an NMDA type receptor hypofunction due to variation, overexpression, or deficiency of gene of constitutive proteins or relevant proteins of an NMDA type receptor in both of the training session and the testing session of the reference memory task.
6. A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises a substance selected by a screening method as set forth in any one of claims 1 to 5 as an active ingredient.
7. A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises a serotonin 5-HT 1A antagonist selected by a screening method as set forth in any one of claims 1 to 5 as an active ingredient.
8. A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises a choline acetylase inhibitor selected by a screening method as set forth in any one of claims 1 to 5 as an active ingredient.
9. A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises aricept as an active ingredient.
10. A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises quetiapine as an active ingredient.
11. The therapeutic agent for the memory/learning dysfunctions by schizophrenia according to claim 10, which comprises as an active ingredient quetiapine in a daily dose of 5 to 270 mg.
12. The therapeutic agent for the memory/learning dysfunctions by schizophrenia according to claim 10, which comprises as an active ingredient quetiapine in a daily dose of 15 to 90 mg.
13. A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises clozapine as an active ingredient.
14. The therapeutic agent for the memory/learning dysfunctions by schizophrenia according to claim 13, which comprises as an active ingredient clozapine in a daily dose of 0.2 to 34.5 mg.
15. The therapeutic agent for the memory/learning dysfunctions by schizophrenia according to claim 13, which comprises as an active ingredient clozapine in a daily dose of 0.7 to 11.5 mg.
16. A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises as an active ingredient an imide derivative of the formula [1]:



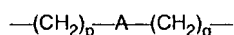
{wherein Z is a group of the formula:



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- 10 (in which B is a carbonyl or a sulfonyl; R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> are independently a hydrogen atom or a lower alkyl, provided that R<sup>1</sup> and R<sup>2</sup>, or R<sup>1</sup> and R<sup>3</sup> may combine each other to form a hydrocarbon ring, or R<sup>1</sup> and R<sup>3</sup> may combine each other to form an aromatic hydrocarbon ring; said hydrocarbon ring may optionally be cross-linked with a lower alkylene or an oxygen atom; said lower alkylene and hydrocarbon ring may optionally be substituted by at least one alkyl; and n is 0 or 1), D is a group of the formula:

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(in which A is a hydrocarbon ring optionally be cross-linked with a lower alkylene or an oxygen atom; said lower alkylene and said hydrocarbon ring may optionally be substituted by at least one alkyl; and p and q are independently 0, 1 or 2),

G is N, CH or COH, and -Ar is an aromatic heterocyclic group, an aromatic hydrocarbon group, benzoyl, phenoxy, or phenylthio, or

G is a carbon atom, and -Ar is a biphenylmethylidene,

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where said aromatic heterocyclic group, aromatic hydrocarbon group, benzoyl, phenoxy, phenylthio, and biphenylmethylidene may optionally be substituted by at least one group selected from a lower alkyl, a lower alkoxy and a halogen atom),

or an acid addition salt thereof.

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17. The therapeutic agent for the memory/learning dysfunctions by schizophrenia comprising as an active ingredient the imide derivative or an acid addition salt thereof according to claim 16, wherein Ar is an aromatic heterobicyclic group, naphthyl, benzoyl, phenoxy or phenylthio, and G is N, CH or COH, or -Ar is a biphenylmethylidene, and G is a carbon atom (said aromatic heterobicyclic group, naphthyl, benzoyl, phenoxy, phenylthio and biphenylmethylidene may optionally be substituted by at least one group selected from a lower alkyl, a lower alkoxy and a halogen atom).

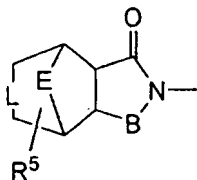
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18. The therapeutic agent for the memory/learning dysfunctions by schizophrenia comprising as an active ingredient the imide derivative or an acid addition salt thereof according to claim 16, wherein Ar is an aromatic heterocyclic group condensed with a benzene ring, or naphthyl, benzoyl, phenoxy or phenylthio (said aromatic heterocyclic group condensed with a benzene ring, naphthyl, benzoyl, phenoxy, and phenylthio may optionally be substituted by at least one group selected from a lower alkyl, a lower alkoxy and a halogen atom), and G is N, CH or COH.

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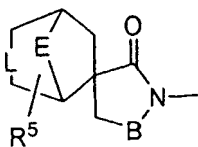
19. The therapeutic agent for the memory/learning dysfunctions by schizophrenia comprising as an active ingredient the imide derivative or an acid addition salt thereof according to claim 16, wherein Z is a group of the formula:

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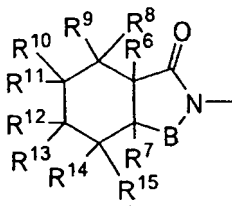


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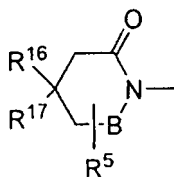
55 (in which -L- is a single bond or a double bond, E is a lower alkylene optionally substituted by a lower alkyl, or an oxygen atom, R<sup>5</sup> is a hydrogen atom or a lower alkyl, and B is the same as defined in claim 14); a group of the formula:



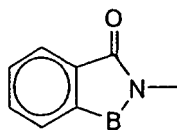
(in which -L-, E, R<sup>5</sup> and B are as defined above);  
a group of the formula:



(in which R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup> are independently a hydrogen atom or a lower alkyl, or the adjacent two groups of R<sup>6</sup>, R<sup>7</sup>, R<sup>8</sup>, R<sup>9</sup>, R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup> may combine each other to form a double bond, and B is as defined above);  
a group of the formula:

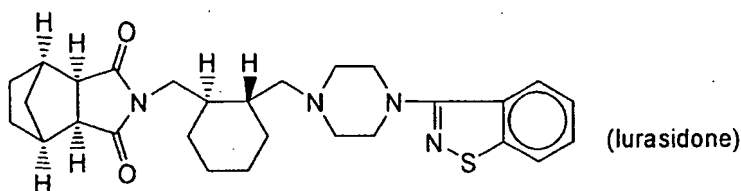


(in which R<sup>16</sup> and R<sup>17</sup> are independently a hydrogen atom or a lower alkyl, or R<sup>16</sup> and R<sup>17</sup> may combine each other to form a saturated hydrocarbon ring, and R<sup>5</sup> and B are as defined above); or  
a group of the formula:

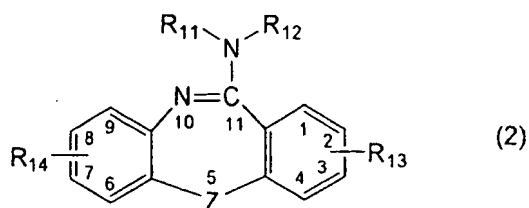


(in which B is as defined above).

20. A therapeutic agent for the memory/learning dysfunctions by schizophrenia comprising as an active ingredient the imide derivative or an acid addition salt thereof, wherein the compound of the formula [1] is lurasidone:



21. A therapeutic agent for the memory/learning dysfunctions by schizophrenia, which comprises as an active ingredient a compound of the formula (2):



wherein Z is a divalent sulfur, imino, or lower alkylimino;

R<sub>11</sub> is a hydrogen atom or an alkyl having 1 to 5 carbon atoms;

R<sub>12</sub> is a hydrogen atom, an alkyl having 1 to 5 carbon atoms, a phenyl, an R<sub>15</sub>-substituted phenyl, an aminoalkyl having 1 to 5 carbon atoms, a lower alkylaminoalkyl having 2 to 8 carbon atoms, a lower alkylamino, an amino, or a lower alkylamino; or

R<sub>11</sub> and R<sub>12</sub> may combine each other together with N to form a 1-pyrrolidinyl, piperidino, morpholino, thiomorpholino, 1-piperazinyl, a 4-lower alkyl-1-piperazinyl, a 4-(hydroxy-lower alkyl)-1-piperazinyl or a 4-(lower alkoxy-lower alkyl)-1-piperazinyl; and

R<sub>13</sub>, R<sub>14</sub>, and R<sub>15</sub> are independently a hydrogen atom, a halogen atom, a hydroxy group, a trifluoromethyl, a lower alkyl, a lower alkoxy, or a lower alkylthio, or an acid addition salt thereof.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2005/002838

## A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.<sup>7</sup> G01N33/50, A61K31/445, 31/496, 31/551, 31/554, 45/00,  
A61P25/28, 43/00, C07D211/32, 243/10, 281/14, 417/12, G01N33/15

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl.<sup>7</sup> G01N33/48-98, G01N33/15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Jitsuyo Shinan Koho 1922-1996 Jitsuyo Shinan Toroku Koho 1996-2005  
Kokai Jitsuyo Shinan Koho 1971-2005 Toroku Jitsuyo Shinan Koho 1994-2005

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
JSTPlus (JOIS)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Enomoto et al., 'Dobutsu Model o Mochiita Ko Seishin'byoyaku no Kaihatsu', Brain Science, Vol.25, No.5 (2003), pages 437 to 444	1-5
X	Ebihara et al., 'Togo Shicchosho no Dobutsu Model', Igaku no Ayumi, Vol.208, No.3 (2004 Nen 1 Gatsu), pages 138 to 142	1-5
X	JP 2003-135074 A (Japan Science and Technology Corp.), 13 May, 2003 (13.05.03), (Family: none)	1-5

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search  
31 May, 2005 (31.05.05)

Date of mailing of the international search report  
14 June, 2005 (14.06.05)

Name and mailing address of the ISA/  
Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

Form PCT/ISA/210 (second sheet) (January 2004)

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2005/002838

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Saji, 'Shinkei Kairomo Keisei to Kofunsei Synapse Kasosei ni Kansuru Kodogakuteki Kenkyu-NMDA Juyotai Kaiba Knockdown Dobutsu no Togo Shicchoshoyo no Ninchi Shogai', Shinkei Kairomo Keisei Shogai no Bunshi Kiko ni Kansuru Kenkyu, Heisei 14 Nendo Sokatsu-Buntan Kenkyu Hokokusho, pages 13 to 20 (2003)	1-5

Form PCT/ISA/210 (continuation of second sheet) (January 2004)



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2005/002838

**Box No. II** Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III** Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1 - 5

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP2005/002838

Continuation of Box No. III of continuation of first sheet (2)

Main invention: claims 1-5  
 2nd invention : claim 9  
 3rd invention : claims 10-12  
 4th invention : claims 13-15  
 5th invention : claims 16-20  
 6th invention : claim 21

The special technical feature of the invention of claims 1-5 is "in vivo screening method for estimating whether or not a test compound has the possibility of ameliorating any memory/learning dysfunction by schizophrenia, which method includes the step of evaluating memory/learning functions with the use of a model with NMDA type receptor dysfunction as an animal model of schizophrenia and with the use of reference memory problems".

With respect to claims 9-21, it is already known that NMDA receptor is associated with schizophrenia (dementia praecox) and that substances capable of acting on the NMDA receptor ameliorate cognitive ability (see WO 2002/022581 A, especially description in the column "Background of the Invention").

Consequently, the special technical feature of the invention of claim 9 is "therapeutic agent for memory/learning dysfunction by schizophrenia, comprising aricept as an active ingredient". The special technical feature of the invention of claims 10-12 is "therapeutic agent for memory/learning dysfunction by schizophrenia, comprising ketiapin as an active ingredient". The special technical feature of the invention of claims 13-15 is "therapeutic agent for memory/learning dysfunction by schizophrenia, comprising clozapin as an active ingredient". The special technical feature of the invention of claims 16-20 is "therapeutic agent for memory/learning dysfunction by schizophrenia, comprising the general formula [I] of claim 16 as an active ingredient". The special technical feature of the invention of claim 21 is "therapeutic agent for memory/learning dysfunction by schizophrenia, comprising the general formula (2) of claim 21 as an active ingredient".

Among these inventions, there is no technical relationship involving one or more of the same or corresponding special technical features. Therefore, it does not appear that these inventions are linked with each other so as to form a single general inventive concept.

# REFERENCES CITED IN THE DESCRIPTION

*This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.*

## Non-patent literature cited in the description

- *Science*, 1999, vol. 285, 1870-1874 [0002]
- *Brain Res Rev*, 2003, vol. 41, 268-287 [0002]
- *Psychopharmacology*, 2003, vol. 169, 215-233 [0002] [0003] [0003]
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- *Brain Res*, 1996, vol. 706, 227-232 [0006]
- *Pharmacol Biochem Behav*, 2001, vol. 69, 585-593 [0006]
- *Cell*, 1999, vol. 98, 427-436 [0006]
- *J Neurosci*, 2001, vol. 21, 750-757 [0006]